

## Response of a Pearly Eye Melon Fly *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) Mutant to Host-Associated Visual Cues

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**Abstract.** We report on a pearly eye mutant (*PEM*) line generated from a single male of *Bactrocera cucurbitae* collected in Kapoho, Hawaii. Crossing experiments with colony wild-type flies indicate that the locus controlling this trait is autosomal and the mutant allele is recessive. Experiments with females to assess response to visual oviposition cues (shape and color) suggest that *PEM* flies are at least unresponsive to color, and likely also unable to perceive visual shape cues. This phenotype has been described from field collection before, but its visual abilities have not previously been tested. The rediscovery of the *PEM* phenotype and results of the vision test support the hypothesis that the *PEM* trait has significant negative fitness consequences in the field, and that the recessive allele resulting in this phenotype probably occurs at a low frequency in nature.

**Key words:** color, shape, mark-release-recapture, genetic marker, SIT

*Bactrocera cucurbitae* (Coquillett) (melon fly) is an important pest species in Hawaii, introduced in 1895 (Back and Pemberton 1918). It is considered oligophagous, breeding predominately on cucurbits, and has considerable invasive potential (Duyck et al. 2004, Dhillon et al. 2005). Despite its relatively long life and generally wide host range, eradication of this species from the Okinawa Islands was effective, mainly via an integrated pest management approach that included utilization of the Sterile Insect Technique (SIT) (Koyama et al. 2004).

Eye mutants of *B. cucurbitae* have been found or created over the years. Naturally occurring “yellow-eyed” melon flies were described by Kobayashi et al. (1973). A “light eye” (*le*) mutant was later produced by exposing wild-type laboratory lines to ethyl methanesulfonate (Saul and

McCombs 1992), and a “white eye” (*we*) mutant was reported from exposure to a cobalt-60 source, as well as a spontaneous “yellow eye-2” (*ye-2*) mutant (McCombs et al. 1996, Peabody et al. 2009). Harris et al. (2006) reported on the field discovery of a “Pearly eye” mutant (*PEM*) from ivy gourd (*Coccinia grandis* (L.)) collected in Laie, Oahu island. Both *PEM* and *ye-2* were determined to be autosomal recessive traits, and both were kept as true-breeding strains in the laboratory for some years.

Genetic markers such as light eyes in melon flies have been suggested to be potentially useful as markers for flies released under an SIT program or for field research, as well as to create genetic sex-sorting systems to increase the efficiency of SIT (McCombs et al. 1996, Harris et al. 2006). Less frequently or carefully as-

sessed is the fitness impact of the genetic traits in question, though the need to assess relative fitness has been mentioned (Harris et al. 2006).

In this study we report on the probable rediscovery of *PEM* from field collections conducted in June 2014 in Kapoho on Hawaii island. We found a single male *PEM* in papaya from that collection. Following Harris et al. (2006), we conducted an experiment to assess the genetic basis of the *PEM* trait for comparison with the original report. All *B. cucurbitae* breeding and rearing was conducted using standard methodology (Vargas 1989). We conducted reciprocal outcrosses starting from a single parental pair of *PEM* and wild-type colony (*NE*) reared *B. cucurbitae* from the research colony at the Daniel K. Inouye US Pacific Basin Agricultural Research Center (DKI-PBARC) in Hilo, Hawaii. Crosses were conducted with single pairs in cubical cages (25 cm x 25 cm x 25 cm). For each set of offspring we allowed full siblings to breed to produce an F2 generation, where we observed a 3:1 phenotypic ratio of *NE:PEM* in both reciprocal crosses (Table 1). This outcome shows that the *PEM* locus is not sex linked, and that the allele encoding *PEM* is recessive. These results are in agreement with the findings of Harris et al. (2006), suggesting that the current line is a probable rediscovery of the *PEM* trait they described.

In addition to the crossing experiments, we tested visual responses in female *PEM B. cucurbitae* compared with wild type *B. cucurbitae* from the DKI-PBARC colony using fruit-mimic spheres to understand the fitness consequences of the *PEM* trait (Piñero et al. 2006). One hundred females of each type were placed in separate 1m<sup>3</sup> cages under a simple roof outdoors at DKI-PBARC between 21 December 2015 and 28 January 2016. Cages were placed 2 m apart to ensure similar lighting conditions. For each of seven experimental

dates (replicates) one yellow and one black Tanglefoot-coated (Contech Enterprises Inc, Victoria BC) fruit mimic spheres (8 cm diameter) were hung from a top-mounted carousel, which was rotated at 2 RPM inside each of the two cages.

Females were sorted on the day of the experiment at 4°C. 100 females of each type were then placed in separate 20-cm powdered flight tubes (Boller et al. 1981), which were introduced to separate cages around 08:30 h. Females were allowed to fly from the flight tubes and potentially become caught on the Tanglefoot-coated spheres for six hours. After this period, the number of non-fliers left in each flight tube was recorded, as were the number of females trapped on each fruit-mimic sphere.

Flight ability was similar between the *PEM* and regular colony flies (Table 2). However, we observed a much lower proportion of the *PEM* females on either of the spheres (on average 6 of 94 flying individuals) compared with *NE* flies (average 50 of 97 flying females). Comparing the spheres, we found an average three times as many *NE B. cucurbitae* on yellow spheres compared with black, a significant difference (one-tailed paired *t* test,  $t=5.38$ , d.f. = 6,  $p < 0.002$ ). For the *PEM* females there was no statistically significant difference in the low number of flies caught in the yellow versus black spheres (one-tailed paired *t* test,  $t = 1.216$ , d.f. = 6,  $p = 0.270$ ). Full details are in Table 2.

We conclude from the visual test that the *PEM* line is unable to see colors (due to equivalent catches on spheres of each color), and probably unable to respond to visual shape cues also (overall low number on the spheres). Light detection through ocelli likely occurs in *PEM* flies, as they successfully left the flight tube at about the same rate as the wild-type *B. cucurbitae*. However, it is clear that they did not respond to visual cue stimulation

**Table 1.** Reciprocal single-pair crosses of Pearly Eyed Mutant (*PEM*) and wild-type colony (*NE*) *B. cucurbitae*.  $\chi^2$  goodness of fit tests to a 3:1 *NE:PEM* ratio in the F2 generation of the heterotypic crosses confirm that the mutant allele is recessive (i.e. observed ratio does not differ from 3:1 expectation): *PEM* (m) x *NE* (f) F2  $\chi^2 = 0.207$ , d.f. = 1,  $p = 0.65$ ; *NE* (m) x *PEM* (f) F2  $\chi^2 = 0.01$ , d.f. = 1,  $p = 0.75$ .

Cohort	Generation	Phenotype cross (m x f)	No. pupae	NE-m	NE-f	PEM-m	PEM-f	Phenotype ratio (NE:PEM)
1	P	PEM x PEM	0	0	0	1	1	-
	F1		23	0	0	12	17	0:29
	F2		272	0	0	36	52	0:88
2	P	PEM X NE	0	1	0	0	1	-
	F1		81	39	39	0	0	78:0
	F2		383	31	30	9	9	61:18
3	P	NE x PEM	0	0	1	1	0	-
	F1		76	36	33	0	0	69:0
	F2		311	32	29	12	10	61:22
4	P	NE x NE	0	1	1	0	0	-
	F1		89	41	42	0	0	83:0
	F2		440	45	40	0	0	85:0

**Table 2.** Flight test (FT) results and number of female *B. cucurbitae* caught on yellow and black fruit-mimic spheres after six hours. The overall number of pearly eyed mutants (*PEM*) leaving tubes was similar to wild type (*NE*), but many fewer were caught on spheres, and there was not an increased number on yellow versus black spheres as observed with *NE* females. Response is calculated as (proportion of fliers captured on spheres)\*(proportion of sphere capture on yellow).

Replicate	NE FT	PEM FT	NE Yellow	NE Black	NE Response	PEM Yellow	PEM Black	PEM Response
1	91	86	47	26	0.52	1	7	0.01
2	97	93	46	2	0.47	4	2	0.04
3	93	90	48	27	0.52	5	11	0.06
4	100	99	36	1	0.36	4	2	0.04
5	100	97	33	1	0.33	1	3	0.01
6	96	94	30	11	0.31	0	4	0.00
7	99	97	23	17	0.23	2	0	0.02
Mean (SD)	97 (4)	94 (5)	38 (10)	12 (12)	0.39 (0.11)	2 (2)	4 (4)	0.03 (0.02)

like the wild type colony flies did. We anecdotally observed *PEM* flies aggregating at the bottom of the cage, though no flight ability difference was detected from the flight tube assays.

Since visual stimuli are thought to be particularly important in this species, there is likely to be a very large fitness cost to the *PEM* trait in the field (Prokopy and Owens 1983, Piñero et al. 2006). The fact that the trait has been found at least twice under natural conditions suggests that the recessive allele persists in the wild population at low frequency, preserved in heterozygotes (hybrid protection). In any case, our results suggest *PEM* would not be a suitable marker for SIT or field research, because these *B. cucurbitae* would have a difficult time finding host fruit or mates.

The *PEM* line described here might be genetically distinct from the one described by Harris et al. (2006), though our results suggest that it is not. Despite being found on a different Hawaiian Island, the line in this study was also found to be an autosomal recessive trait, and shares the phenotype of the original *PEM*. Additionally, the current line also changes from pearly to tan after death like the original, again suggesting that they are produced by the same locus. This is in contrast to *ye*, which remained unchanged after death, and *le*, which was reported to darken in dead flies. Certainty that the previous and current *PEM* lines are the same is not possible without direct sequencing of both mutants.

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